



Tightly shut: flexible valve margins and microstructural asymmetry in pteriod bivalves

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Abstract

An organic-rich columnar prismatic outer shell layer, which extends far beyond the underlying nacre, has allowed pteriod bivalves (the pearl oysters and their allies) to develop flexible valve margins, allowing a tight hermetic seal when shut. In some taxa, the microstructural arrangement is known to be asymmetrically developed between the two valves. The asymmetry was surveyed across 29 taxa of pteriods (including representatives of known genera) confirming that it is typically the right valve which has a greater expanse of prism-only shell (and less nacre) and showing that this portion of the right valve has more organic content (more than twice the value in some instances) than the equivalent in the left. A more detailed investigation of prismatic material in *Pteria penguin* comparing the right and left valves revealed that the right valve flange has a higher density of smaller prisms, each with its organic envelope, and not a greater thickness of the organic envelopes themselves. The flange is also thinner on the right valve and shown here to be very flexible when wet. This allows it to bend against the rigid left valve when the shell is closed. Comparison of this structural asymmetry in the pteriods with five outgroup taxa in the Ostreidae and Pinnidae suggests that clades with the asymmetry have been freed from the constraints of a flattened valve morphology and to develop inequivalved forms.

Introduction

Molluscan shell is a biocomposite in which a stiff mineralised phase (usually calcite or aragonite) is embedded in a softer organic matrix that allows energy dissipation, toughness and flexibility (Currey 1999; O'Toole-Howes et al. 2019; Strag et al. 2020). Shells typically are made of multiple microstructural arrangements, arranged in layers, each

having distinctive hierarchical arrangements of the mineral and organic phases (Taylor et al. 1969, 1973; Bieler et al. 2014). Different microstructural types have different properties in relation to not only mechanics but also other factors, such as resistance to dissolution (Chadwick et al. 2019). For any particular taxon, the precise microstructures used for layers are genetically fixed, but the relative thickness of them may be subject to ecophenotypic variation (Telesca et al. 2019). The evolution of particular microstructures and their combinations are presumably under strong evolutionary control, balancing selective advantages and their metabolic costs.

There has been considerable interest in the mechanical performance of biocomposites, in particular of nacre which is well known for its toughness (Jackson et al. 1988; Barthelat et al. 2006; Lin and Meyers 2009; Gim et al. 2019; Sun and Bhushan 2012), and the ways in which they might inform biomimetic designs (e.g. Kakisawa and Sumitomo 2012; Waqar et al. 2018). But despite it being a very early microstructure (Taylor 1973; Vendrasco et al. 2019), many extant molluscan groups do not secrete nacre and even where it does occur, it is always accompanied by at least one other microstructure (usually external to it) (Cartwright and Checa 2007; Bieler et al. 2014). In the bivalves, which show an

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extraordinary diversity of microstructural arrangements (Taylor et al. 1969, 1973; Bieler et al. 2014), nacre is often accompanied by an outer layer of aragonite prisms in the Palaeoheterodonta and the Anomalodesmata or by calcite prisms in Pteriomorphia. There remains much to understand about the adaptive significance of different microstructural arrangements and the overall aim of this paper is to explore this in relation to the pteriod bivalves.

A common feature of epifaunal bivalves is the possession of flexible valve margins that allow tight hermetic sealing along a broad flat flange around the edge of the shell (Vermeij 1987). This character is found in a range of taxa but principally in the Pteriomorphia within the order Ostreida which are characterised by the possession of an outer shell layer of calcite prisms perpendicular to the valve surface (Bieler et al. 2014; Lemer et al. 2016) (Fig. 1). In the living bivalve, these margins are very flexible and allow it, upon adduction, to close the shell with a substantial flange of material from both valves juxtaposed rather than just at the very margins. The excellent hermetic seal which it allows may have a range of advantages such as protection against predators or against ingress of harmful substances or loss of fluids (Vermeij 1987), in particular through desiccation during emersion in intertidal taxa (Harper and Skelton 1993). Additionally, the ability to form a tight seal after injury allows repair even after quite a catastrophic shell damage (Vermeij 1983; Dietl and Alexander 2005).

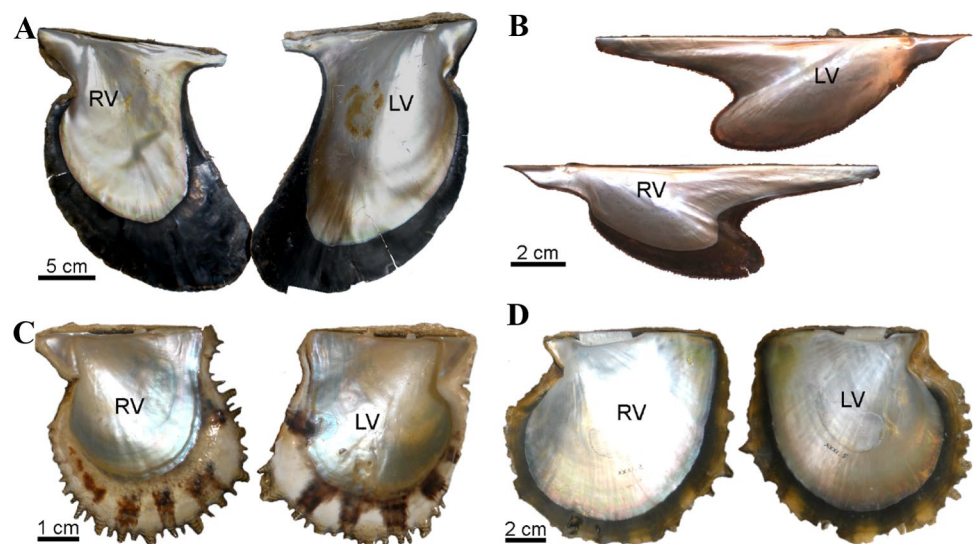
Bivalves with flexible valve margins typically construct them from microstructures with a relatively high percentage of organic matrix between the crystals. Here we explore the form of the flexible valve edges in the Order Ostreida, in particular in the Pterioidea. The group is interesting because unlike most bivalves where the microstructural arrangement is the same in the two valves, in many pteriod taxa there is a marked asymmetry whereby the inner layers are less

laterally extensive in one valve and also the thickness of the outermost layers appears to differ between them.

The Pterioidea are a clade of shallow-water tropical and subtropical bivalves that exploit a range of epifaunal modes of life, some in intimate commensal relationships with sponges, hydroids and alcyonarians (Tëmkin 2006a). It includes the well-known ‘pearl oyster’ genera *Pinctada* and *Pteria* which are of considerable commercial importance. The clade has a long evolutionary history, dating back to the Ordovician (Pojeta and Runnegar 1985), with extinct members of the clade including the Pterineidae and Bakevelliidae, and have been major players in the autecological category of exposed byssate bivalves (Skelton et al. 1990).

Traditionally, the extant Pterioidea have been thought to comprise four families: the Pteriidae, Pulvinitidae, Isognomonidae and Malleidae. While the superfamily is always recovered as a monophyletic group in both morphological and molecular phylogenetic analyses (Steiner and Hammer 2000; Giribet and Distel 2003; Tëmkin 2006a, 2010; Bieler et al. 2014; Combosch et al. 2017), there has been much less support for recognising the constituent families as monophyletic (excluding the Pulvinitidae which is monospecific). Whereas studies based on both morphology and molecular data have failed to support the traditional placement of genera within families, perhaps the most significant finding in molecular phylogenies has been consistent dismantlement of the family ‘Pteriidae’ as a clade comprising both *Pteria* and *Pinctada* (Tëmkin 2010; Lemer et al. 2016; Combosch et al. 2017). It is also notable that although earlier morphological analyses suggested the Pinnoidea as sister-group to the pteriods e.g. (Waller 1978; Giribet and Wheeler 2002) with similarities in microstructure being amongst key evidence, a more exhaustive morphological analysis (Tëmkin 2006a) and various molecular analyses have consistently shown the Ostreioidea in that role (Steiner and Hammer 2000; Bieler

Fig. 1 Internal views of selected species of pteriods. **a** *Pteria penguin* (Noumea, New Caledonia), MNHN Paris. **b** *Pteria crocea* (Caubian Island, Philippines), UGR. **c** *Pinctada fucata* (Japan), MNHN Paris. **d** *Pinctada margaritifera*, original in Ranson (1961). (Mahé Island, Seychelles), MNHN Paris. LV left valve, RV right valve



et al. 2014; Combosch et al. 2017). This suggests that the apparent similarities between the pinnoids and pteroids are merely convergent.

The valve microstructures of pteroids follow the same basic pattern. They have two microstructural types: an outer layer of columnar calcite prisms and an inner layer of sheet nacre made of aragonite, which is traversed by the pallial myostracum (Bieler et al. 2014). The nacreous layer does not extend to the valve edges, leaving a single layer of calcite prisms in the most ventral zone. The microstructure of this outer, more extensive, shell layer consists of relatively coarse columnar prisms (up to 150 μm in width in some cases) secreted perpendicular to the valve surface. With growth, selection occurs among individual prisms so that smaller prisms progressively disappear and larger ones increase and become more similar in width as the layer thickens. Individual prisms are separated from one another by relatively thick organic envelopes $\sim 0.5\text{--}3\ \mu\text{m}$ wide (Checa et al. 2005, 2016). One notable characteristic of the prismatic flange in living individuals is its extreme flexibility, but in dried specimens in museum collections, it becomes inflexible and very brittle (Tëmkin 2006a). As noted above, another key feature of this flexible prismatic flange in pteroids is that it is often more extensive (i.e. wider) on one valve than the other. This asymmetry has been used by Tëmkin (2006a) as a morphological character for phylogenetic analysis and is clearly apparent in Fig. 1a–c.

In the current study, we use the recent molecular phylogeny of the Pterioidea (Tëmkin 2010) for investigating the occurrence of flexible margins within the Pterioidea. Specifically, we seek to (1) investigate differences in the extent of prismatic flange and its organic content between taxa and also between different valves of the same individual to explore the occurrence of asymmetry; (2) explore the flexibility of prismatic material in a hydrated state; (3) consider the phylogenetic and possible adaptive significance of these traits.

Materials and methods

Materials

We examined representative taxa of extant pteroids in the collections of the Zoology Museum of the University of Cambridge (UMZC), and the Muséum National d'Histoire Naturelle, Paris (MNHN), and the mollusk research collection housed in the Department of Stratigraphy and Paleontology, University of Granada (UGR). In all, we were able to investigate species from each of the extant genera of all recognised families (including most of the type species). We concentrated on the 'pearl oysters', i.e. members of the genera *Pteria* and *Pinctada* and in particular on the taxa

included in the most comprehensive molecular phylogeny of the pearl oysters (Tëmkin 2010). Additionally, we studied members of all three extant genera of Pinnoidea and representatives of some oysters as representatives of outgroups. All taxa used in this study are listed in Table 1.

Morphometric measurements

For all individuals observed, the following measurements were made with digital calipers: maximum dimension (dorsoventral diameter) of the whole bivalve and, for each valve separately, the distance of the ventral margin of the nacre from the hinge along the direction of maximum growth (see Fig. 2). From these data, we calculated the ratios LV/RV to capture the degree of symmetry in microstructure and determined the linear extent of the prismatic flange for each valve as a proportion of the entire valve. Where possible we made measurements for multiple individuals (over as wide a range of size as possible) of a particular species to obtain mean values but this was not possible in all instances. Although less than ideal, the use of smaller numbers appears justified because for those instances where ten individuals were available for study (e.g. *Pinctada margaritifera* and *Pteria colymbus*) there were no significant differences ($p > 0.05$) for the proportion of asymmetry over the range of sizes available. An exception, however, appears to be *Electroma alacorvi*, where of five individuals there was a greater asymmetry in the larger individuals although our sample size was not sufficient to demonstrate the significance of this.

Optical and scanning electron microscopy

Selected shell pieces were examined by optical and scanning electron microscopy (SEM). For optical microscopy, we used a Nikon SMZ1000 binocular microscope equipped with a digital image acquisition system. Specimens observed in SEM (in high vacuum mode) were previously cleaned with commercial bleach ($\sim 5\%$ active chlorine), ultrasonicated and air dried, followed by coating with carbon (Hitachi UHS evaporator) and investigated using an Environmental SEM (ESEM) FEI Quanta 400 and a FEG-ESEM FEI QUENCH-SCAN 650F (Center for Scientific Instrumentation, CIC, University of Granada, UGR, Spain).

For two specimens of *Pteria penguin* (MNHN 927796, shell length 201 mm, and MNHN 983231, shell length 152 mm) estimates were made of prism density and the thickness of the organic membranes between individual prisms for the prismatic margins of both left and right valves. For each sample, rectangular units (approximately $15\text{--}20 \times 7\text{--}8\ \text{mm}$) from exactly the same areas of both valves were cut out from the ventral prismatic margins. The six positions determined on each bit were evenly distributed on the sample surface and were coincident on both valves

Table 1 List of material examined and measured parameter values (as defined in Fig. 2)

Taxon studied, with repository and locality details	N	Extent of nacre as a proportion of dorsoventral diameter, recorded as a ratio L/R mean value (SD where N > 5)	Extent of prismatic flanges as a proportion of dorsoventral diameter for both left and right valve left/right
'Pteriidae'			
<i>Pinctada margaritifera</i> (Linnaeus, 1758) MNHN: Vietnam, French Polynesia, Philippines, Red Sea; UGR: French Polynesia	10	0.99 (0.03)	0.11/0.12
<i>P. albina</i> (Lamarck, 1819) MNHN: Philippines, W. Australia	10	1.09 (0.04)	0.15/0.21
<i>P. capensis</i> (Sowerby III, 1890), Ranson plate 22; MNHN: Seychelles	1	1.05	0.06/0.10
<i>P. chemnitzii</i> (Philippi, 1849) MNHN: Torres Strait	1	1.17	0.23/0.35
<i>P. fucata</i> (Gould, 1850) MNHN: Japan	1	1.15	0.21/0.25
<i>P. imbricata</i> Röding, 1798 MNHN: Red Sea	10	1.11 (0.09)	0.17/0.25
<i>P. radiata</i> (Leach, 1814) MNHN: Red Sea, Gulf of Aden, Bermuda; UGR: Philippines, Senegal	10	1.11 (0.04)	0.16/0.24
<i>P. maculata</i> (Gould, 1850) MNHN: French Polynesia	5	1.04 (0.08)	0.10/0.14
<i>P. maxima</i> (Jameson, 1901) MNHN: Torres Strait	1	1.09	0.18/0.25
<i>P. mazatlanica</i> (Hanley, 1856) MNHN: Pacific coast of Mexico and Panama	10	1.00 (0.02)	0.11/0.12
<i>P. nigra</i> (Gould, 1850) MNHN: Vietnam	1	1.01	0.36/0.36
<i>Pteria avicular</i> (Holten, 1802) UGR: Philippines	4	1.28	0.04/0.24
<i>Pt. colymbus</i> (Röding, 1798) MNHN: Gulf of Mexico	10	1.20 (0.10)	0.16/0.26
<i>Pt. heteroptera</i> (Lamarck, 1819) UGR: Philippines	1	1.56	0.02/0.38
<i>Pt. lata</i> (Gray in Eyre, 1845) MNHN: Loc. unknown	1	1.34	0.13/0.38
<i>Pt. penguin</i> (Röding, 1798) MNHN: French Polynesia; UGR: Philippines	8	1.22 (0.08)	0.13/0.29
<i>Pt. dendronephthya</i> Habe, 1960 UGR: Philippines	1	1.22	0.13/0.29
<i>Isognomon perna</i> (Linnaeus, 1767) MNHN: Loc. unknown	2	1.02	0.36/0.37
<i>I. ephippium</i> (Linnaeus, 1758) MNHN: Loc. unknown	1	1.00	0.35/0.35
<i>I. isognomon</i> (Linnaeus, 1758) MNHN: French Polynesia, Red Sea; UGR: Philippines	9	1.22 (0.28)	0.31/0.42
<i>I. janus</i> Carpenter, 1857 MNHN: Loc. unknown	1	0.99	0.39/0.38
<i>I. legumen</i> (Gmelin, 1791) MNHN: Red Sea	2	1.40	0.04/0.30
<i>I. nucleus</i> (Lamarck, 1819) MNHN: Loc. unknown	1	1.02	0.19/0.21
<i>Electroma alacorvi</i> (Dillwyn, 1817) 1MNHN: Philippines, French Polynesia	5	1.07 (0.05)	0.35/0.40
<i>Crenatula picta</i> (Gmelin 1791) MNHN: Suez	3	1.01	0.37

Table 1 (continued)

Taxon studied, with repository and locality details	N	Extent of nacre as a proportion of dorsoventral diameter, recorded as a ratio L/R mean value (SD where N > 5)	Extent of prismatic flanges as a proportion of dorsoventral diameter for both left and right valve left/right
<i>Vulsella vulsella</i> (Linnaeus, 1758) MNHN: Philippines	5	0.99 (0.01)	0.20
Malleidae			
<i>Malleus albus</i> Lamarck, 1819 UGR: Philippines	2	1.00	0.64
<i>M. malleus</i> (Linnaeus, 1758) UGR: Philippines	2	1.02	
Pulvinitidae			
<i>Pulvinites exempla</i> (Hedley, 1914)	1	1.01	0.34/0.35
Outgroup comparisons			
Ostreidae			
<i>Ostrea edulis</i> Linnaeus, 1758 UGR: Southern Spain	5	NA	0/0.14
Pinnidae			
<i>Atrina pectinata</i> (Linnaeus, 1767) UGR: Philippines	1	0.98	
<i>Atrina vexillum</i> (Born, 1778) UGR: Philippines	2	1.00	
<i>Pinna bicolor</i> Gmelin, 1791 UGR: Philippines	1	0.98	
<i>Streptopinna saccata</i> (Linnaeus, 1758) MNHN: Red Sea	1	1.00	0.73/0.73

N=number of specimens. Data for *Pulvinites exempla* derived from figured specimens (Tëmkin 2006b). Species authority references are provided in the Electronic Supplementary Information

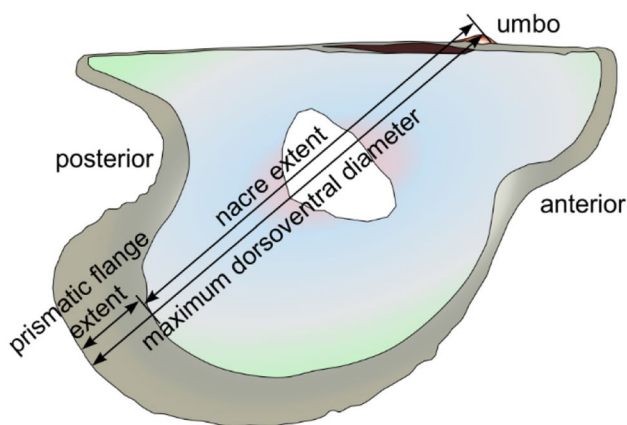


Fig. 2 Sketch of interior of an idealized left valve of *Pteria* species, showing dimensions measured on specimens

when the two internal surfaces opposed, i.e., abutted when the valves were shut (see Supplementary Information for details). The number of prisms was counted within an area $415 \times 280 \mu\text{m}$. Only complete (i.e. not cut by the edge of the image) prisms were counted. We also estimated inter-prism membrane thickness based on four measurements along

transects across the micrographs. The mean thickness of the prismatic lamellae was also assessed.

Thermogravimetric analysis (TGA) to determine organic content

The organic content of samples of the prismatic flanges of shells was determined using a thermogravimetric analyser (METTLER-TOLEDO TGA/DSC1) of the CIC (UGR). Briefly, the weight loss of very finely ground small pieces (mostly 40–45 mg original weight) of air-dried shell material (original weight from 20–50 mg) were constantly recorded (precision = $0.1 \mu\text{g}$) over a temperature range between 35–38 °C and 950 °C (heating rate = $10 \text{ }^\circ\text{C min}^{-1}$). Left and right valves of the same individuals were sampled in all cases and the material was carefully cleaned to remove any traces of periostracum or other adherent material. Shell was removed from the most ventral portion of the margin. Significant weight losses between 200 °C (when both free and structural water losses were complete) and 600 °C (when CaCO_3 decomposition into CaO begins) were attributed to the combustion of organic matter (Harper et al. 2009).

Investigation of flexed valves

To investigate the geometry of the flexible margin when the valves were shut, two specimens of *Pinctada radiata* and one of *Pteria avicular* from the MNHN collections were selected. They were chosen because they had clearly died when the adductor muscle was fully contracted and remained tightly shut with tissue desiccated in situ. These individuals were embedded in clear polyester resin (Vosschemie, GTS), sectioned along the maximum growth direction and polished to 1 μm .

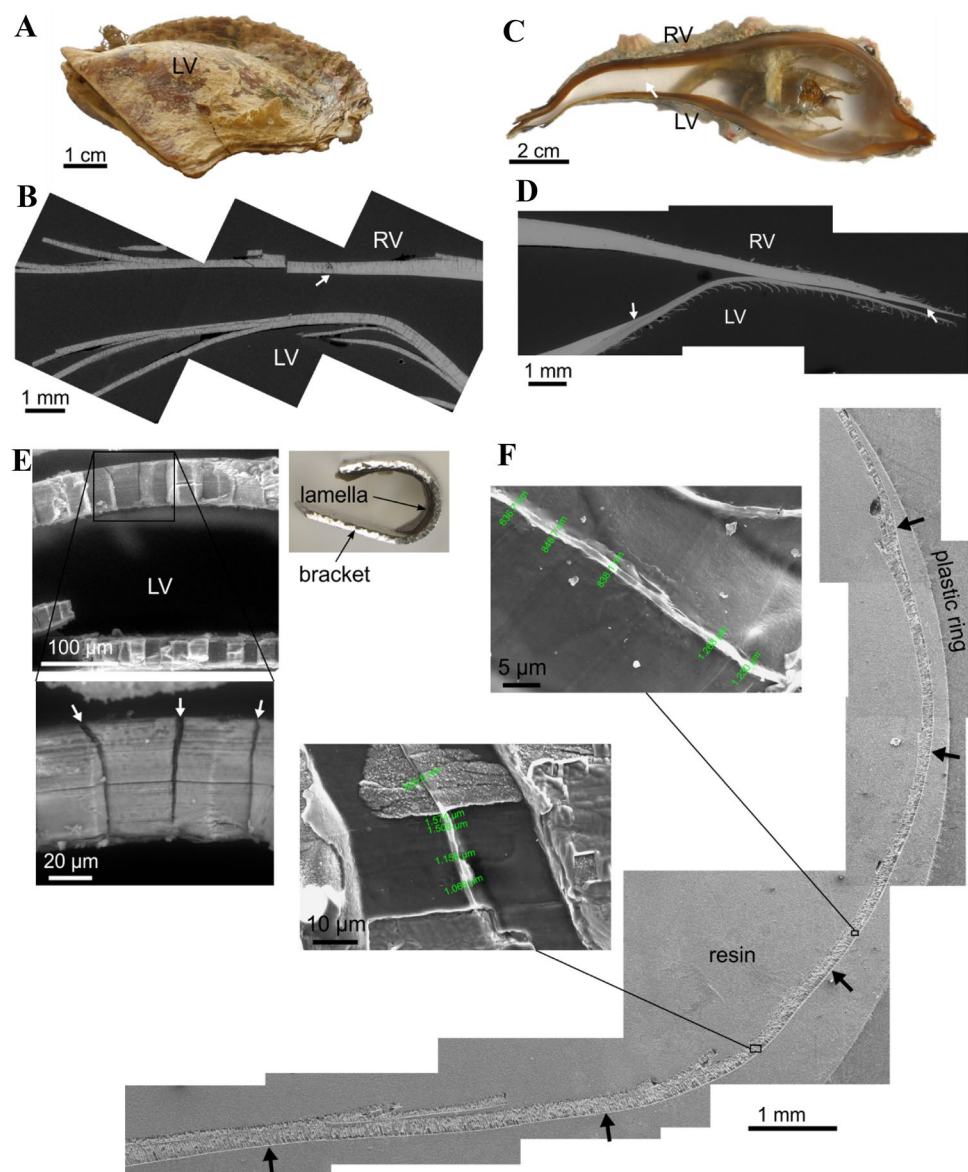
We also investigated the flexibility of the material when fully hydrated by cutting pieces of prismatic calcite and soaking them in water. When flexible, the pieces were flexed along an approximately co-marginal axis, and inserted into the interior of aluminium or plastic brackets (see Fig. 3e).

Some samples were later examined in the environmental mode in an Environmental SEM (ESEM) FEI Quanta 400 of the CIC (UGR). Others were embedded while still hydrated in resin, sectioned, polished (to 0.25 μm), and etched with 5% EDTA for two min. The surfaces were coated with carbon (Hitachi UHS evaporator) and observed in a FEG-ESEM FEI QUEMSCAN 650F (CIC, UGR).

Results

Table 1 presents the summary results for the extent of the prismatic margin and degree of asymmetry between valves in each species studied. Two patterns emerge. Firstly, the extent of the ‘prismatic flange’ is greatest in the Isognomnidae, Malleidae and Pinnoida where the nacre routinely

Fig. 3 Flexed margins in pterioids. **a** *Pinctada radiata* (Borj Djillidj, Djerba, Gulf of Gabés, Tunisia), MNHN Paris. **b** SEM composite micrograph of sectioned margins of a specimen of *Pinctada radiata* (Borj Djillidj, Djerba, Gulf of Gabés, Tunisia), MNHN Paris. **c** Dorsioventral section of *Pteria avicular* (Tatu Hiva, French Polynesia), MNHN Paris. **d** SEM composite micrograph of sectioned margins of a specimen of *Pteria avicular* (Andol Point, Caubian Island, Philippines). Arrows in **b** and **d** indicate maximum extension of internal nacre layer. Specimens **a** to **d** died with valves fully addressed against each other. **e** Rehydrated prismatic lamella of a specimen of *Pinctada radiata* (Olango Island, Philippines) forced to flex within a metallic bracket (right image). Upper SEM image is a close up of folded lamella, while lower image shows stretched organic membranes on convex side of lamella (arrows). **f** Rehydrated prismatic lamella of *Pteria hirundo* (littoral of Málaga, Spain) flexed within a plastic ring, resin-embedded, polished and etched. Two details show an increase in thickness of organic membranes towards convex internal surface (as in natural conditions). LV left valve, RV right valve



extends to less than three-quarters of the maximum length of the shell, and is often much less (e.g. *Crenatula picta* and *Streptopinna saccata*), whereas in most pterioidea examined, the nacre is more extensive, i.e. the prismatic flange is less extensive. However, even in the pterioidea, the prismatic margin is a significant portion of the valve. Secondly, for some taxa, most notably all *Pteria* species examined, some *Pinctada* and some *Isognomon* (*I. isognomon* and *I. legumen*), there is a high degree of asymmetry (taken here to be instances where the metric comparing the two valves is > 1.10) in the shell microstructure between the right and left valves. Typically, where there is an asymmetry, it is the right valve which has the greater amount of exposed prisms. In only those taxa for which we had more than ten specimens, we investigated whether there was any systematic link between valve size and the degree of asymmetry displayed but no cases were discovered.

TGA results

Table 2 shows the results for percentage weight loss in the temperature range 200–600 °C. Although it was not practicable to perform replicates for each sample, to check the accuracy of the measurements, we made two replicates on the same powdered samples of both valves of the *Pteria avicular* in Table 2. We compared % weight losses at four different temperatures (200, 300, 450 and 600 °C) and found differences of 0.007–0.22%, which indicates a good replication of the results. We also checked differences between two different samples obtained from both valves of a single specimen, namely *Pinctada margaritifera* 2 (Table 2), at the same temperatures. Differences ranged between 0.04–0.87%, which implies little variability, depending on where the prismatic flange was sampled, but the overall results are consistent between samples.

For some of the taxa (*Malleus malleus*, *Vulsella vulsella*, *Atrina vexillum* and *Pinna bicolor*) there was comparatively little difference between the organic contents of the right

Table 2 Weight loss between 200 and 600 °C for samples taken from prismatic flanges of left and right valves of study species

Taxon	Max dimensions (mm)	% weight loss right valve	% weight loss left valve
Pterioidea			
<i>Pinctada margaritifera</i> 1	78	6.69	4.54
<i>P. margaritifera</i> 2	250	6.36	6.44
		6.66	6.60
<i>P. margaritifera</i> 3	78	4.34	3.97
<i>P. margaritifera</i> 4	64	4.36	6.24
<i>Pinctada radiata</i> 1	75	7.02	5.91
<i>P. radiata</i> 2	52	7.95	6.88
<i>P. radiata</i> 3	63	6.57	4.67
<i>P. radiata</i> 4	49	5.42	4.60
<i>P. radiata</i> 5	85	11.80	8.11
<i>Pteria penguin</i> 1	212	8.22	4.92
<i>P. penguin</i> 2	212	10.60	6.50
<i>P. penguin</i> 3	202	14.20	8.01
<i>P. penguin</i> 4	201	13.40	5.82
<i>Pteria colymbus</i>	Not recorded	9.59	7.64
<i>Pteria avicular</i> 1	72	8.38	16.04
<i>P. avicular</i> 2	58	4.91	7.76
<i>Pteria dendronephthya</i>	87	11.4	5.37
<i>Pteria hirundo</i>	85	8.54	4.83
<i>Isognomon isognomon</i>	207	5.03	4.05
<i>Crenatula picta</i>	37	3.96	6.09
<i>Malleus malleus</i>	99	2.56	2.75
<i>Vulsella vulsella</i>	83	3.56	3.90
Pinnoidea			
<i>Atrina vexillum</i>	Not recorded	3.52	3.00
<i>Pinna bicolor</i>	Not recorded	3.50	3.58

and the left valves. However, for all *Pteria* spp., *Pinctada radiata*, *Pinctada margaritifera* (except for the very largest individual) and *Crenatula picta* there were marked differences ($<0.25\%$) between the values recorded from the left and right valves of the same individual with those for the right valve being routinely around twice the value of the left. Only *Pteria avicular* (both individuals) and single individuals of *Crenatula picta* and *Pinctada radiata* presented the opposite case, with the left valves having more organics.

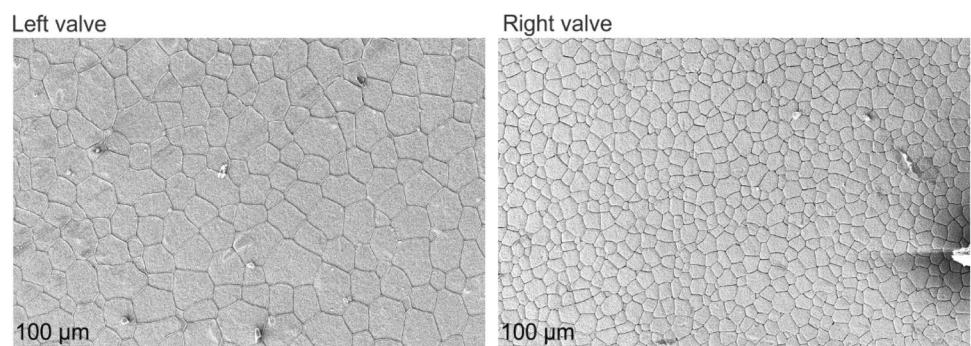
Observations on sections through conjoined valves

Figure 3b–d illustrates the sawn and polished blocks of *Pinctada radiata* and *Pteria avicular*, respectively. These clearly show that the marginal parts of the valves are brought into direct contact forming an extensive flange. They also show that the right valve at this point is much thinner than the left. There is a marked asymmetry in the flexure, such that it is the thinner right valve which is clearly bent against the left, and that the nacre in the left valve acts as a rigid foil.

Table 3 Number of prisms and membrane and shell thicknesses in equivalent positions of left and right valves in *Pteria penguin*. See “Materials and methods” and Supplementary Information for details

Specimen 983231					
Position	Left valve		Right valve		LV/RV thickness
	No. of prisms	Membrane thickness (nm)	No. of prisms	Membrane thickness (nm)	
1	158	700–850	714	Not present	1.46
2	185	600–700	835	650–800	
3	83	700–1000	564	Not present	
4	90	750–1100	652	Not present	
5	79	900–1100	1145	500–700	1.58
6	115	850–1100	359	750–950	
Specimen 927796					
Position	Left valve		Right valve		LV/RV thickness
	No. of prisms	Membrane thickness (nm)	No. of prisms	Membrane thickness (nm)	
1	3420	550–2150	1046	2100–3300	1.12
2	476	750–1000	1756	450–1050	
3	581	650–1200	1102	850–1250	
4	426	500–1050	3674	400–900	
5	515	650–1300	751	550–2000	1.01
6	362	850–1050	637	850–1700	

Fig. 4 Internal surfaces of two valves of a specimen of *Pteria penguin* (Talibon, Bohol province, Philippines), taken at a point where valves approximately contact each other. Density of prisms is markedly higher in right valve



Scanning electron microscopy

For both of the individuals of *Pteria penguin* for which we measured the density of prisms for standard areas, in five of the six replicates (exception was Specimen 927796, position 1), the number of prisms for the right valve was greater than for the left (Table 3) (see example in Fig. 4; all images in Supplementary Information). The difference between the number of prisms in both valves in Specimen 983231 is marked, whereas in Specimen 927796 this difference is attenuated. The one exception might be explained if the prismatic lamella of the left valve was in a very incipient stage of growth, typically characterized by many small prisms, which, with thickening of the lamella, become progressively fewer and larger (the so-called prism competition; e.g. Checa et al. 2016). The thickness of interprismatic membranes varied between 400 and 2150 nm but with no systematic differences discernible between those of left and right valves.

The extreme flexibility of the prismatic material was shown by the ability to bend the wet lamellae within metal brackets (Fig. 3e). Examination of these flexed samples using SEM (environmental mode) provided a good illustration of how the organic membranes expand in the convex zone (Fig. 3e), where the material is in tension. In embedded, polished and etched specimens, this feature is more difficult to appreciate due to the 2D nature of the sections. Nevertheless, they reveal the high degree of flexing the material can reach without fracturing (Fig. 3f). Although we have not carried out a proper statistical study, measurements made on individual organic membranes also reveal consistent increments in thickness towards the convex surface (Fig. 3f). Our results are not surprising since it is only the organic phase which provides the necessary deformation upon bending.

Discussion

Our survey agrees with Tëmkin (2006a) in reporting that the prismatic margins of a number of pteroid taxa are asymmetrically developed, with a tendency for a greater expanse of prismatic flange on the right valve (see Tëmkin 2006a, character 104). Moreover, our data suggest that the prismatic material of the two valves differs, with that of the right being more organic-rich. In the more detailed inspection of *Pteria penguin*, it was observed that the higher organic content of the prismatic layer of the right valve was the result of an increased number of smaller prisms, each with its own organic envelope, and not by any increase in the thickness of organic membranes per se. In taxa such as *Pinctada radiata* and *Pteria avicular*, where the prismatic flange of the left valve is very short, when the valves are shut the more extensive right flange meets the nacreous portion which provides a non-pliable counterpart (Fig. 3b, d).

The organic contents reported herein are arguably amongst the highest levels of organic material of all molluscan microstructures thus far analysed (higher only in Supplementary Information of Checa et al. 2016, up to 16% were reported in a pinnoid). High flexibility of valve margins, whether asymmetric or not, is conferred by high organic content as has been noted for pinnoids (Checa et al. 2016) and the lyonsiid *Entodesma navicula* (7.4%; Harper et al. 2009), although it is clear that this flexibility only occurs in fresh individuals where the organic material is fully hydrated; O'Toole-Howes et al. (2019) note a two-fold difference in the Young's Modulus between wet and dry samples of similar organic material from bivalve shells. It is clear that thick organic walls between prisms stretch and compress during flexing, as demonstrated by our observations on re-hydrated specimens (Fig. 3e, f). Our data show that in those taxa where there is an asymmetric development of the prismatic margin, the organic content of the more extensive right valve is typically higher than that of the left and it is clear that it is this valve which flexes against the more rigid left valve. Additionally, the right valve is usually thinner, which also reduces its resistance to flexing.

This trend towards microstructural asymmetry has been achieved independently several times within the pteriomorphs and even within the Pterioidea. Figure 5 is a plot of our findings on the molecular phylogeny of the pearl oysters published by Tëmkin (2010). It should be noted that Tëmkin identified the 'imbricata' complex as containing a number of units which have been variously identified as species (e.g. *Pinctada imbricata*, *P. fucata* and *P. radiata*), whose relationships are unclear and made problematic by their morphological variability, widespread geographic distribution and their transport and hybridization through the culture of pearls.

Figure 5 reveals that asymmetry occurs in a number of different clades: all *Pteria*, perhaps two different ones in *Pinctada* (the more basal taxa *P. longisquamosa* and *chemnitzii* and the 'imbricata' complex [though this pattern might be interpreted as a character which reverses in the intervening taxa] and also within the Isognomonidae [*Isognomon isognomon* but not *I. ephippium*]). These results underline the parallelism and convergence which is rife in the pterioids (Tëmkin 2006a). It is also of note that the outgroup, the ostreoids, also displays asymmetry of valve microstructure with development of a prismatic margin in just the right valve (Taylor et al. 1969; Carriker et al. 1980) suggesting convergent development of a similar trait.

The multiple acquisition of this asymmetry suggests that there may be some adaptive advantages to this derived state. It seems clear that the perceived advantages of flexible margins in general and their capacity to form a tight seal (Vermeij 1987) still apply for the asymmetric arrangement. What, then, might be the advantages of such an asymmetric

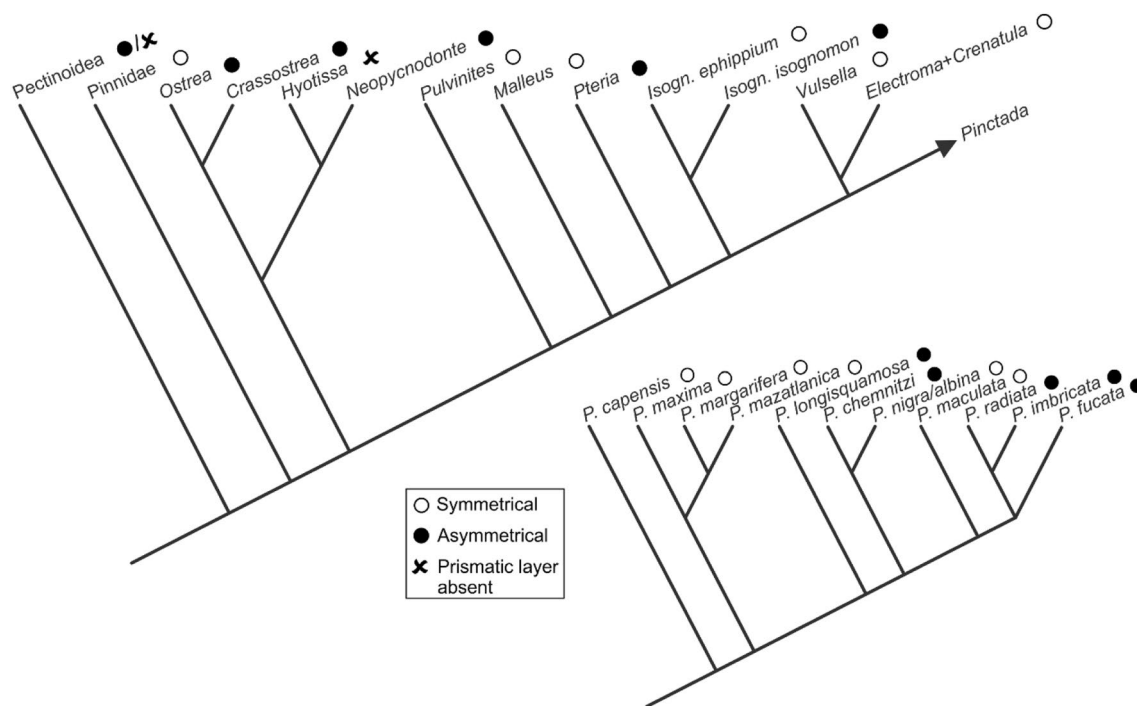


Fig. 5 Distribution of asymmetric and symmetric microstructure plotted on molecular phylogenetic relationships of Tëmkin (2010). *Pinctada longisquamosa* scored from Mikkelsen et al. (2004, p. 480)

development? Possession of flattened shells with a low width between the valves may be particularly useful in taxa that nestle in restricted crevices or between the branches of alcyonarians, for example. Such morphologies, however, restrict an organism's internal space for viscera, most notably perhaps restricting space for gills and reproductive organs. A consequence of the asymmetry between valves described herein, and the ability of one valve to flex against the other may be that it allows the bivalve to develop a more inequivalve form which is principally exploited in the more pleurothetic life habits. It is interesting to note that it is almost invariably the right valve which has the broader prismatic flange and consequently it is the left valve which becomes more cup shaped—which tends to be 'uppermost' in the pteriods. In the oysters, the more cupped, less flexible valve, is the 'lowermost'. There have been multiple evolutionary pathways to bivalves exploiting different adaptive strategies which may be interpreted as anti-predatory (Vermeij 1983, 1987; Harper and Skelton 1993). A key recurrent strategy has been to reduce the danger of marginal damage (Vermeij 1983). The ability to form a tight hermetic seal is likely to be a critical part of the pteriod armour against predators in several different ways, for example, in sealing the bivalve from initial detection by potential predators, by forming a 'sacrificial rim' on the outside of the shell and, if damage is not fatal, sealing tightly allowing the enhanced possibility of regrowth and repair without attracting secondary predators.

The superposition of the highly flexible prismatic layer with its ability to stop cracks (Strag et al. 2020), and the nacre which is very tough (Wegst et al. 2015; Gim et al. 2019) but which at the same time is very resistant to bending (e.g. Furuhashi et al. 2009) has created a versatile armour that is effective against a range of predatory taxa with different attack strategies (Vermeij 1987; Harper and Skelton 1993).

Lemer et al. (2016) regard the simple prismato-nacreous shell structure of pteriods and pinnoids as having limited their life habit repertoire compared with other pteriomorphs that had evolved the foliated shell microstructure. Nevertheless, we suggest that the combination of the two microstructures has produced effective armour. The development of the asymmetry described here in the pteriods also enabled morphological novelty, and a broader range of life habits, including becoming pleurothetic and lying on one valve.

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Data availability statement All data are available in the Tables—raw TGA data—available from the authors upon reasonable request.

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflicts of interest.

Ethical approval No live individuals were captured as part of this study.

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